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TITLE: ALTERATIONS IN SKELETAL MUSCLE MICROCIRCULATION OF HEAD-DOWN TILTED RATS

AUTHORS:

Bernhard Stepke[1], Ph.D., John T. Fleming, Ph.D., Irving G. Joshua, Ph.D. and X.J. Musacchia, Ph.D.

LABORATORY OF ORIGIN:

Department of Physiology and Biophysics, University of Louisville, Louisville KY 40292

ADDRESS FOR CORRESPONDENCE:

Bernhard Stepke, Ph.D.

Department of Physiology

University of Missouri

Columbia MO 65212

Phone (314) 882 7666 or 882 4957

RUNNING HEAD: Microcirculation in 0 g

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[1]Dr. Stepke is currently a post-doctoral fellow in the department of Physiology, University of Missouri, Columbia MO 65212.

ABSTRACT

In this study we assessed the function of microscopic blood vessels in skeletal muscle (cremaster muscle) for alterations which may contribute to the observed elevation of blood pressure associated with head-down tilted whole body suspension (HDT/WBS), a model of weightlessness. Arteriolar baseline diameters, vasoconstrictor responses to norepinephrine (NE) and vasodilation to nitroprusside (NP) were assessed in control rats, rats suspended for 7 or 14 day HDT/WBS rats, and rats allowed to recover for 1 day after 7 days HDT/WBS. Neither baseline diameters nor ability to dilate were influenced by HDT/WBS. Maximum vasoconstriction to norepinephrine was significantly greater in arterioles of hypertensive 14 day HDT/WBS rats. This first study of the intact microvasculature in skeletal muscle indicates that an elevated contractility of arterioles to norepinephrine in suspended rats, and suggests an elevated peripheral resistance in striated muscle may contribute to the increase in blood pressures among animals subjected to HDT/WBS.

INDEX WORDS: Blood pressure, Norepinephrine, Nitroprusside, Microcirculation, Cremaster muscle, Head-down tilt, Microgravity

INTRODUCTION

The head-down tilted whole body suspended (HDT/WBS) rat is widely accepted as a model for responses seen during or following weightless flight (1,9,11,14,16,17,19,20). Much of the earth-side model experiments dealing with muscle and bone responses have corroborated space flight results. However, studies of the cardiovascular and circulatory responses have been less confirmatory (9) and require more intensive experimentation.

Previous work with the HDT/WBS rat revealed an elevation in mean arterial blood pressure (9,14). The elevation in blood pressure among the suspended rats could result from elevations in cardiac output, total peripheral resistance or both. There have been no systematic investigations of changes in cardiac output or total peripheral resistance to indicate which factor may be more contributory to the development of hypertension. An elevated total peripheral resistance is characteristic of most models of hypertension. Thus it is reasonable to consider that an elevated peripheral resistance contributes to the development of hypertension among suspended rats. Increased vascular resistance may result from functional alterations such as enhanced reactivity to endogenous constrictor agonists and/or structural alterations.

Data from several studies support the contention that vascular changes occur in the suspended rat, particularly in the hindquarter, which is composed mostly of skeletal muscle. An increase in iliac artery resistance has been observed at 24 hours

(19) and 72 hours (7) of suspension. After 9 days of suspension (14), iliac artery blood flow velocity (inversely proportional to hindquarter vascular resistance) decreased more among suspended rats than in control rats during submaximal exercise, a physiological condition associated with an increase in sympathetic drive. These observations raised the possibility that an increase in striated muscle vascular resistance, perhaps induced by adrenergic receptor stimulation, may contribute to an increase in mean arterial blood pressure. Therefore our study was designed to investigate possible changes in the small resistance arterioles of skeletal muscle (the predominant tissue of the hindquarter) among suspended rats. We used the well established cremaster muscle model which manifests functional alterations in early stages of various forms of experimental hypertension (5,4,13).

In this study, comparisons were made of the basal diameters of resistance arterioles in the cremaster muscle of control (non-suspended) rats and rats which had been suspended for seven days, fourteen days or allowed to recover for one day after seven days of suspension. Arteriolar reactivity to exogenous norepinephrine (NE) was assessed since this vasoconstrictor is known to play an important role in the regulation of blood pressure. Finally, the dilator responses to nitroprusside were assessed as an indicator of the degree of basal tone of the arterioles.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River, Wilmington MA) weighing approximately 170 grams were allowed to acclimate to laboratory conditions for at least five days. Systolic blood pressure was estimated by tail artery plethysmography in the conscious rats. Then they were assigned to one of 4 experimental groups: control, seven day suspended, fourteen day suspended or one day recovery from seven days of suspended.

Whole-body Suspension Procedure:

The suspension method has been previously described in detail by Musacchia et al. (12). Each rat was lightly anesthetized with pentobarbital (25 mg/kg i.p.), then fitted with a custom tailored whole-body cloth suit and suspended at a head-down tilt of 20° from the horizontal. Free movement of fore- and hindlimbs was possible, but only the forelimbs could be used for body positional adjustments. The animal movements included some side to side movement to access water and rat chow ad libitum. After seven or fourteen days, each rat was removed from suspension. One day recovery rats were removed from the suspension apparatus after seven days of suspension and returned to a cage for normal ambulatory movement for a period of one day before experimentation.

On the day of the microvascular experiment, the conscious rat was placed in a heated (37°C) restrainer. Measurements of tail artery systolic blood pressure and heart rate were obtained with the use of an occluder cuff and pneumatic transducer. The

rats were then anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed on a heating pad to maintain rectal temperature at 36.0-37.5°C. Tracheal cannulation (to maintain airway) and carotid artery cannulation (to monitor blood pressure) were performed.

Microvascular Experiments:

The right cremaster muscle was prepared as described previously (4). The scrotum was opened and the cremaster muscle was incised longitudinally. The muscle was outstretched over a cover slip in a 50 ml plexiglass bath using sutures. The bath was then filled with a physiologic salt solution (PSS) containing: NaCl, 113 mM; $NaHCO_3$, 25 mM; dextrose, 11.6 mM; KCl, 4.7 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; KH_2PO_4, 1.2 mM; (osmolality: 285 mOsm/kg H_2O). The temperature of the bath PSS was maintained at 34.5±0.5°C by a feedback system connected to an indwelling heating coil. The bath pH was maintained at 7.40±0.05 by aerating the solution with carbon dioxide.

The rat was then placed on the stage of a microscope and the microvessels of the cremaster muscle were visualized by transilluminated light. A closed-circuit television system displayed the microvessel images on a video monitor, and a video recorder stored the images for later analysis. The magnification of the image was determined by displaying a stage micrometer scale on the monitor screen. Actual in situ vessel diameters were then calculated using the magnification factor. The experimental

protocol was begun after a one hour equilibration period to allow for recovery from surgical manipulations.

Based on branching order and size, the first- (A1), a second- (A2; 60-90μ), and a third-order arteriole (A3; 10-20μ) were identified and selected for observation. A single A3 having a resting diameter of 10-15 microns, which subsequently branched to form capillaries, was selected.

Experimental Protocol:

Basal diameters and dilation to nitroprusside

Baseline diameters of the selected vessels were measured twice (five minutes apart) then nitroprusside was added to the tissue bath to establish a bath concentration of 10^{-5} M to maximally dilate the vessels. The arteriole diameters were measured after 10 minutes of exposure. The solution was drained from the bath and the muscle washed several times with drug-free PSS to remove the nitroprusside.

Constriction to norepinephrine

After a thirty minute equilibration period, new baseline diameters were measured, then norepinephrine was added to the bath. The diameters of the same arterioles were measured after 10 minutes exposure to multiple bath concentrations (10^{-7} M, $3x10^{-7}$ M, 10^{-6} M, $3x10^{-6}$ M, 10^{-5} M, $3x10^{-5}$ M) of norepinephrine. After the last measurement, the bath solution was drained, and the muscle was washed several times with drug-free PSS. Vessel diameters were again measured after 30 minutes equilibration time. To confirm

preparation viability, dilator responses to nitroprusside (10^{-5} M) were again assessed.

Drug Preparation:

Drugs were freshly prepared for use on the day of experiment. Norepinephrine (Sigma) was prepared in a saline solution containing ascorbic acid solution (1 mg/ml) to retard oxidative degradation. Nitroprusside (Malinkrodt) was prepared in 0.9% saline.

Data Analysis:

An ANOVA was used to compare baseline diameters, minimum and maximum diameters after nitroprusside and norepinephrine, respectively, between the four experimental groups. When statistical significance was noted, Student's t-tests were used. With all tests, a probability of less than 5% ($p<0.05$) was considered statistically significant. Data are presented as mean ± sem, and statistically significant differences are indicated with an asterisk.

RESULTS

Systemic observations include significantly elevated tail artery blood pressure measurements in suspended rats as compared to their own presuspension measurements: in the seven day suspended group (108±7 versus 94±6 mm Hg); fourteen day suspended group (113±5 versus 95±6 mm Hg) and one day recovery group (112±6 versus 92±5 mm Hg). Under pentobarbital anesthesia, the mean arterial pressure of the seven day suspended rats was not significantly higher than that of the controls (116±4 versus 103±4 mm Hg) but was significantly elevated among the fourteen day suspended rats (134±4 versus 103±4; $p<0.05$). One day after recovery from seven days of suspension these rats also showed a significant pressure elevation compared to controls (129±9 versus 103±4 mm Hg; $p<0.05$). These results are summarized in Table 1.

In the microcirculation of the cremaster muscle, the resting baseline diameters of the A1, A2 and A3 arterioles of the 3 experimental groups did not differ significantly from the values of the control (non-suspended) group (Figure 1). The A1 arterioles of the fourteen day suspended rats contracted to a significantly ($p<0.05$) smaller diameter than those of control non-suspended rats in response to norepinephrine (NE). Compared to the 52% constriction of control A1 arterioles, the A1 arterioles of the fourteen day suspended rats constricted by 72%. No differences in A2 or A3 arteriole constriction to NE were noted between the groups.

Nitroprusside did not significantly dilate the A1 and A2 arterioles in any of the experimental groups (Figure 2). Nitroprusside dilated the A3 arterioles from the non-suspended control, seven day and fourteen day suspended animals to comparable values, but did not dilate those A3 arterioles of the one day recovery rat group (NS).

DISCUSSION

Simulated weightlessness models are associated with alterations in body fluid and/or cardiovascular variables (1,9,14,19,20). Musacchia and colleagues reported an elevated mean arterial blood pressure in conscious rats as early as 3 days after whole body suspension in a head-down tilted position, which remained significantly elevated throughout seven days (9,11). Similarly, a modest but significantly elevated mean arterial blood pressure in conscious rats 9 days after suspension has been reported (14). In contrast, no change in mean arterial blood pressure was noted among conscious rats (16) or anesthetized rats (17) after seven days of suspension. Likewise, Brizzee and Walker (1) using a different suspension model (the tail suspended rat), reported no change in mean arterial blood pressure after seven days of suspension. Thus, the observation of elevated blood pressure after suspension has not been a consistent finding. We considered the possibility that the development of hypertension among suspended animals was time-dependent, requiring more than seven days for consistent manifestation. Thus, in this study the suspension period among one group of rats was extended. While conscious, a significant elevation of tail artery blood pressures was observed after seven days of suspension, but following anesthetization, only a trend for increased mean arterial blood pressure was found among these same rats. It is possible that anesthesia normalized blood pressure among the suspended rats as has been shown for other hypertensive rats (18). Yet, after

fourteen days of suspension, both tail artery pressure and mean arterial blood pressure were significantly elevated indicating that this period of suspension evoked overt cardiovascular changes which were not masked by induction of anesthesia. Thus in these experiments, the onset of hypertension among suspended rats appears to require at least fourteen days for consistent development of a modest form of hypertension.

Our direct observations of the intact cremaster muscle microvasculature revealed that the resting diameters of the A1 and A2 arterioles did not significantly differ across groups. Since A3 arterioles of comparable resting diameter were selected from each muscle (rather than measuring the diameter of all A3's in each muscle), it cannot be concluded whether the overall diameters of A3 are altered by the suspension. Unchanged baseline arteriolar diameters have been reported in the cremaster muscle microcirculation of hypertensive rats (7). However, reduced baseline arteriolar diameters has been reported (5,13). Thus reduced baseline arteriolar diameters in the skeletal muscle microvasculature is not a feature of all forms of hypertension. The etiology and duration of the hypertension may determine whether changes in resting arteriolar diameter are observed. With modest hypertension, such as we have detected in the whole body suspended rats, changes in resting arteriolar tone may be masked by the dilatory effect of anesthesia (8). Alternatively, the hypertension observed in the suspended animals may be due to an elevation of resistance in vascular beds other than skeletal

muscle. It is also appropriate to consider that though the diameters are not different between suspended versus control rats under anesthetized resting conditions, with altered conditions (such as stress), the microvessels of suspended rats may exhibit a greater constriction than control animals resulting in an elevation of blood pressure. This concept was also suggested by Overton et al. (14). Measurements of hindquarter blood flow velocity during exercise (15) suggest that hindquarter blood vessels of suspended rats constrict more than non-suspended rats to the stress of exercise. Thus, we reasoned it appropriate to assess arteriolar reactivity to an endogenous vasoconstrictor.

Since significant increases in plasma norepinephrine have been reported after seven (20,21) and fourteen days of suspension (21), and enhanced arteriolar contractility (maximal vasoconstriction) to norepinephrine could then increase vascular resistance. Hence we assessed arteriolar responses to norepinephrine

In seven day suspended rats, which showed only a tendency towards increased mean arterial blood pressure, the A2 arteriole showed only a tendency towards a heightened contractility to norepinephrine (Figure 2). While in the fourteen day suspended rats, which were significantly hypertensive, the A1 arteriole contractility was significantly elevated. Similarly, enhanced norepinephrine contractile responses have been demonstrated in the cheek pouch of renovascular hypertensive hamsters (2) and in the cremaster A3 of one-kidney one-clip hypertensive rats (4,5).

Thus, enhanced contractility to norepinephrine appears to be common to an early developmental stage of suspension-induced hypertension as other classical forms of hypertension. However, the observation of adrenal hypertrophy in the suspended rat (10) suggests that stress may be a contributing component to responses observed in this model.

A3 arteriolar dilation to nitroprusside (Figure 2) revealed comparable maximum diameters for A3 arterioles among all groups indicating that basal tone (maximum diameter minus resting diameter) was not different between groups. Furthermore, A3 dilation dispelled any notion that structural changes had occurred in the walls of the arterioles to limit the dilator constrictor capacities. Such structural changes leading to impaired small arteriolar dilation has been reported to occur within one week following the development of diabetes (3) but is usually not manifested until much later in the development of hypertension (13).

In conclusion, head-down tilted whole-body suspension induces a modest elevation of arterial blood pressure that becomes significant after fourteen days. At this time, the large arterioles of the cremaster microcirculation exhibit an enhanced contractility to exogenous norepinephrine. If such a change takes place throughout the skeletal muscle vasculature and in other vascular beds, total peripheral resistance may be increased during episodes of noradrenergic stimulation and lead to an increase in mean arterial blood pressure among suspended rats.

Acknowledgements:

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TABLE 1: Experimental Rat Body Weights and Blood Pressures

			Tail Artery B.P.(mm Hg)	
	Weight (g)	MAP (mm Hg)	pre HDT	post HDT
Control	166±4 (12)	103±4 (11)	--	89±4 (7)
7d HDT/WBS	156±3 (9)	116±8 (8)	94±6 (4)	108±7▲ (3)
14d HDT/WBS	165±6 (9)	134±4* (9)	95±6 (8)	113±5▲ (8)
1d Recovery	157±3 (9)	129±9* (9)	92±5 (6)	112±6▲ (6)

Mean arterial blood pressure (MAP) of anesthetized rats and tail artery blood pressure (B.P.) using tail artery plethysmography among conscious rats, before and after whole body suspension. Data are expressed as mean ± sem. (*) denotes statistically significant ($p<0.05$) differences from corresponding values from the control group, (▲) denotes statistically significant ($p<0.05$) differences from presuspension measurements. The numbers within the parentheses refer to the number of observations.

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FIGURE LEGENDS

FIGURE 1. Resting baseline and minimum (maximum norepinephrine response) diameters of first (A1), second (A2) and third order (A3) arterioles in control (C), 7 day HDT/WBS (7d), 14 day HDT/WBS (14d) and 1 day recovery from 7 days HDT/WBS (1d). Data are expressed as mean ± sem. Statistically significant ($p<0.05$) differences are denoted by (*).

FIGURE 2. The dilation of first (A1), second (A2) and third order (A3) arterioles to topicqal nitroprusside. The data are expressed as percent change in diameter accomplished by topical nitroprusside stimulation (mean ± sem).

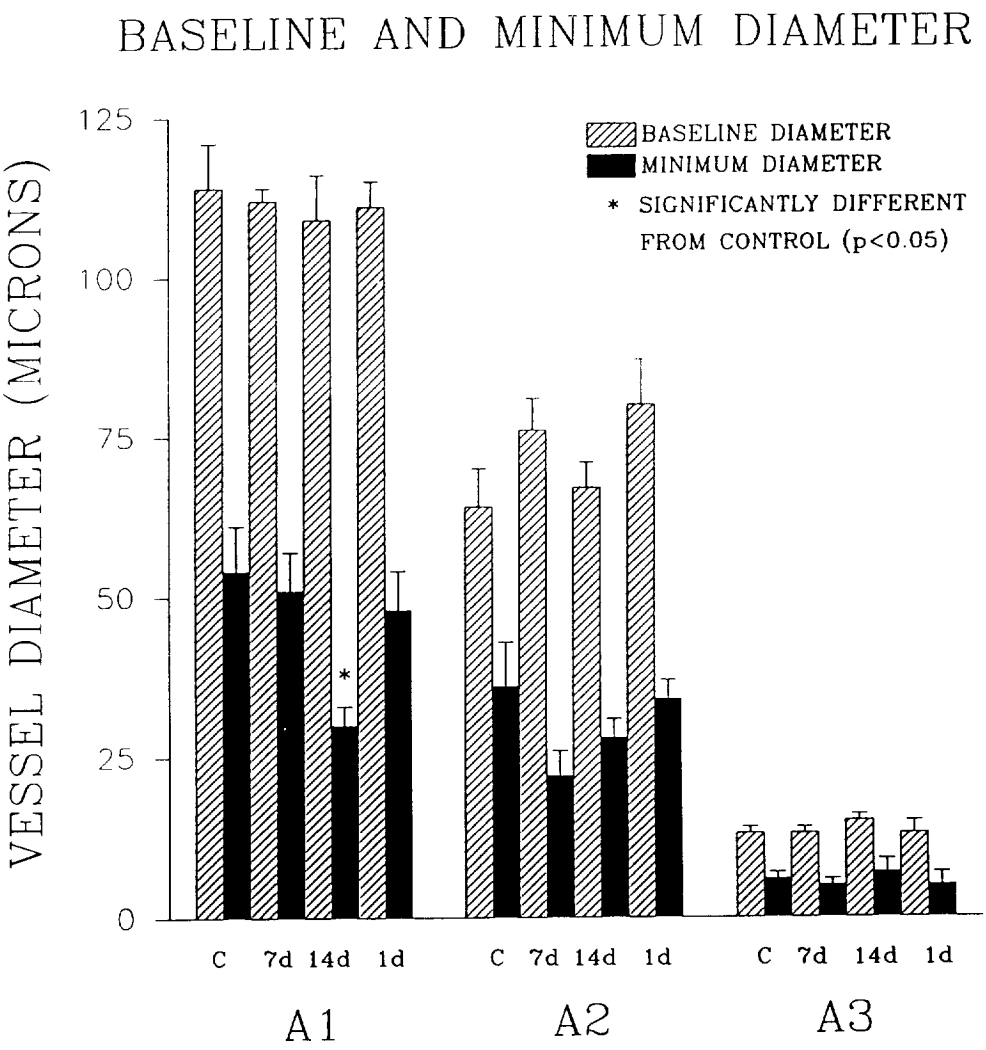
BASELINE AND MINIMUM DIAMETER
VESSEL DIAMETER (MICRONS)
125
100
75
50
25
0
BASELINE DIAMETER
MINIMUM DIAMETER
* SIGNIFICANTLY DIFFERENT FROM CONTROL (p<0.05)
*
C 7d 14d 1d
C 7d 14d 1d
C 7d 14d 1d
A1
A2
A3

Figure 1.

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DILATOR RESPONSE TO NITROPRUSSIDE (10^{-5}M)

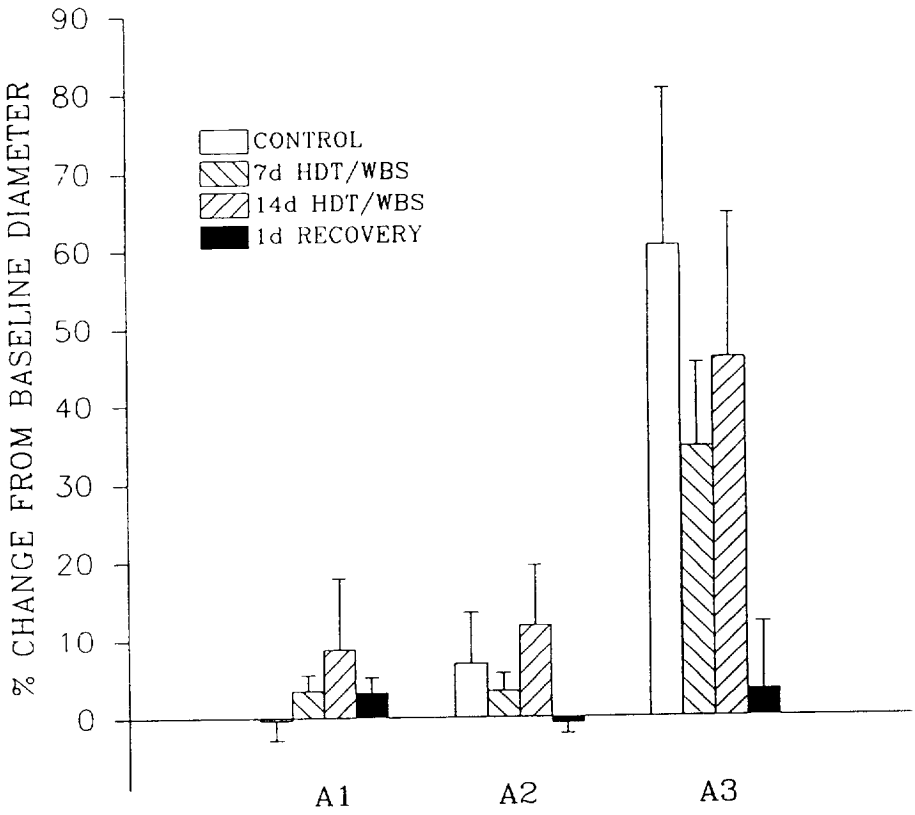

Figure 2.

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